The Effects of Temperature and Daylength on the *Rosa* Polyphenism in the Buckeye Butterfly, *Precis coenia* (Lepidoptera: Nymphalidae)

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Abstract. In North Carolina, *Precis coenia* that emerge during the Summer months exhibit a ventral hindwing (VHW) with well-defined reddish-brown and brown pattern elements on a light tan background. During late Summer and early Fall, however, individuals begin to appear with poorly defined or obscured pattern elements on a dark reddish-brown background. The present study shows that the Fall (*rosa*) color morph can be induced by either low rearing temperatures or short daylengths. The effect of such conditions seems to be cumulative throughout the larval life, although animals are much more sensitive during the last 24 hours of larval life and immediately after pupation.

Introduction

Seasonal polyphenism involves a repeating pattern of changing phenotypes which is under the control of an environmental factor (Shapiro, 1976; Tauber, et. al., 1986). It is important to differentiate this from genetic polymorphism, where the phenotypic differences in a population are the result of genetic differences. The Buckeye butterfly, *Precis coenia* (Lepidoptera:Nymphalidae), has long been known to exhibit a seasonally polyphenic color pattern (Clark, 1932; Klots, 1951; Mather, 1968). The background coloration of the ventral hind wing (VHW) surface changes with the season: the Summer (*linea*) morph has a pale tan background with well-defined pattern elements while the Fall (*rosa*) morph has a dark reddish-brown background with indistinct elements (Figure 1). The *rosa* morph becomes predominant during the Fall and early Winter, with the exact time of its appearance depending on local conditions.

The control of the *rosa* polyphenism had not been studied in detail, though several authors have speculated on its mechanism. Clark (1932) suggested that high humidity played a role while Howe (1975) proposed a photoperiodic control. At the very least, the control mechanism did not seem to be straightforward, a fact which prompted Shapiro (1976) to report no obvious environmental correlations at all. The present study, however, shows that the *rosa* morph can be induced by exposing developing larvae either to low temperatures or short daylengths.

Materials and methods

Precis coenia stock was derived from a laboratory population maintained at Duke University and constituted from animals collected over a period of years in the region around Durham, NC. A random sample of the eggs produced by this
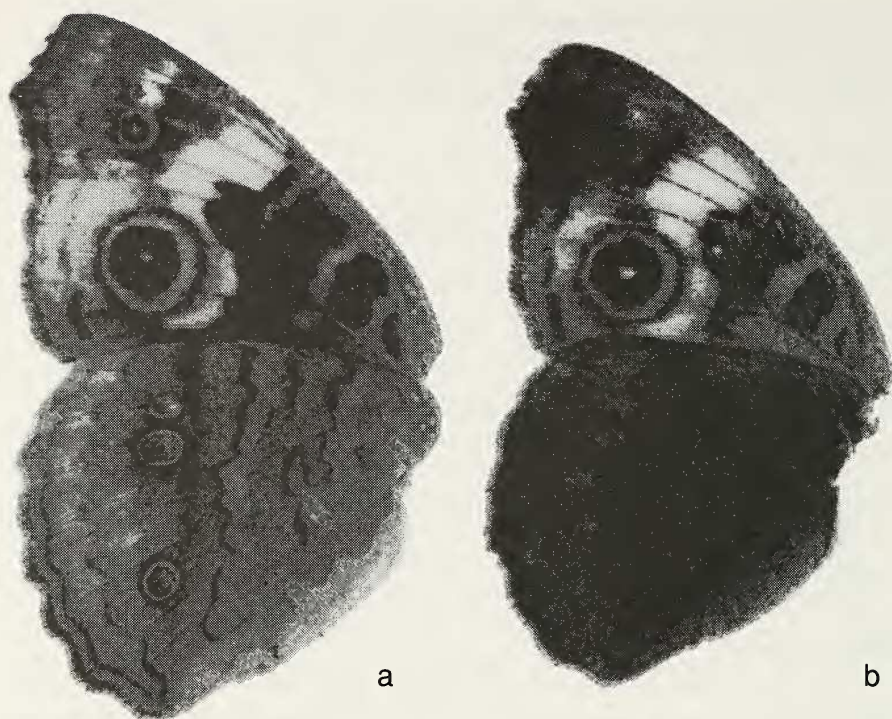
a   b

Figure 1. Ventral wing patterns: 1a) shows the ventral wing pattern of the Summer or *Linea* morph (score = 0). The backgound is light tan and the pattern elements are quite distinct. 1b) shows the ventral pattern of the Winter or *Rosa* morph (score = 3). The background coloration is uniformly reddish-brown and the pattern elements are no longer distinct.

population was used to found a breeding colony which was maintained under controlled environmental conditions at a temperature of 27°C and a daylength of 16 hrs. light : 8 hrs. dark (16L:8D). To assess the influence of daylength and temperature on the expression of the *rosa* morph, eggs were removed from the breeding colony and randomly assigned to one of nine controlled temperature and daylength regimes.

Animals assigned to "seasonal" conditions (from June through November of 1990) were reared in a semi-shaded area of an outdoor cage. Those assigned to regimes of 27°C, 16L:8D were reared in the same constant temperature room as the breeding colony. All other regimes were maintained in incubators (accuracy of ± 1°C) with lighting provided by 15W fluorescent bulbs on clock timers. All larvae were mass-reared in plastic boxes on an artificial diet containing powdered *Plantago lanceolata* leaves (Smith, 1991).

Results

Adults from each experimental treatment as well as from the breeding colonies were scored as to VHW background coloration. The scoring system assigned each animal a score from 0 (the *linea* Summer morph)

to 4 (an extreme *rosa* morph) and is summarized in Table 1. The response of each treatment was then defined in terms of two response characteristics: mean score and % response (percentage of animals with scores > 0).

BREEDING COLONY

Non-*linea* morphs (score > 0) were encountered in the breeding colony. However, the incidence of such morphs was relatively low (14%) and there were no individuals with more than intermediate expression of the

Table 1. Scoring system: System used used to quantify the degree of *Rosa* expression. Mean score and % response (percentage of animals with score > 0) were the two response characteristics used to compare treatments. All animals were scored at least twice and in no case did group means vary by more than 5%.

| SCORE	MORPH	DESCRIPTION
0	*Linea*	Light tan VHW background with distinct reddish-brown pattern elements.
1	Light Intermediate	Predominantly tan VHW background with pinkish tinge proximal to the mid-wing umbral band and along the trailing edge of the hindwing.
2	Dark Intermediate	Predominantly reddish-brown VHW background with tan areas still visible distal to the mid-wing umbral band. Pattern elements in the reddish-brown areas somewhat obscured.
3	*Rosa*	Uniformly reddish-brown VHW background and wing edges with red or pink regions distal to the mid-wing umbral band. Pattern elements severely obscured.
4	Extreme *Rosa*	Solid reddish-purple VHW background with vestigial pattern elements and distal wing edges a dark purple.
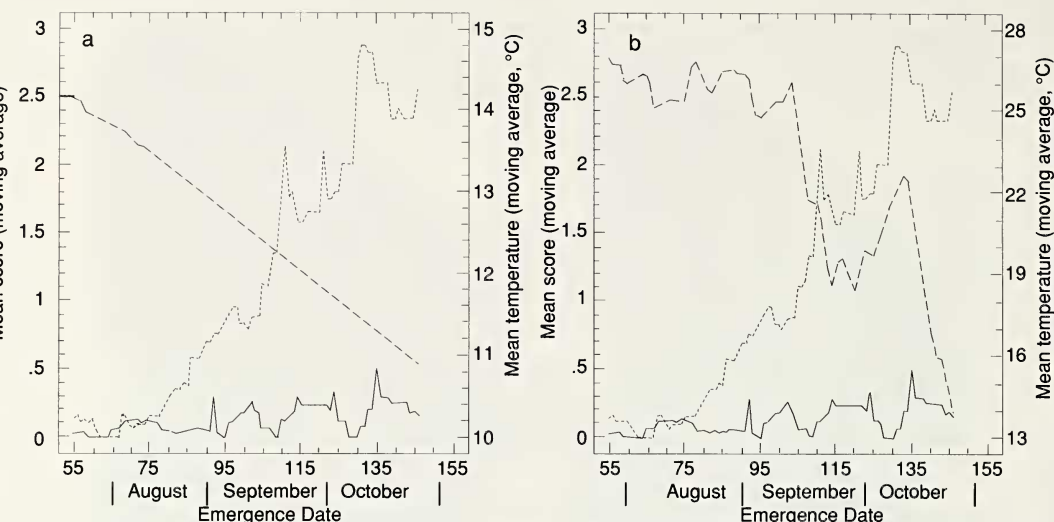
Figure 2. Response to seasonal conditions: The graphs show the moving mean scores (9 day interval centered on date of emergence) of animals raised under seasonal conditions in an outdoor cage as compared to those under the controlled conditions of the breeding colony (27°C, 16L:8D). The mean scores of the two groups are initially quite similar but begin to diverge in mid-August and become maximally dissimilar by October. 2a shows the response of the two groups as compared to the seasonal daylengths. 2b shows the response of the two groups as compared with the moving mean (9 day interval) of average daily temperatures.

—————— Breeding colony scores (27°C, 16L:8D)

- - - - - - seasonal colony scores (outdoor cage)
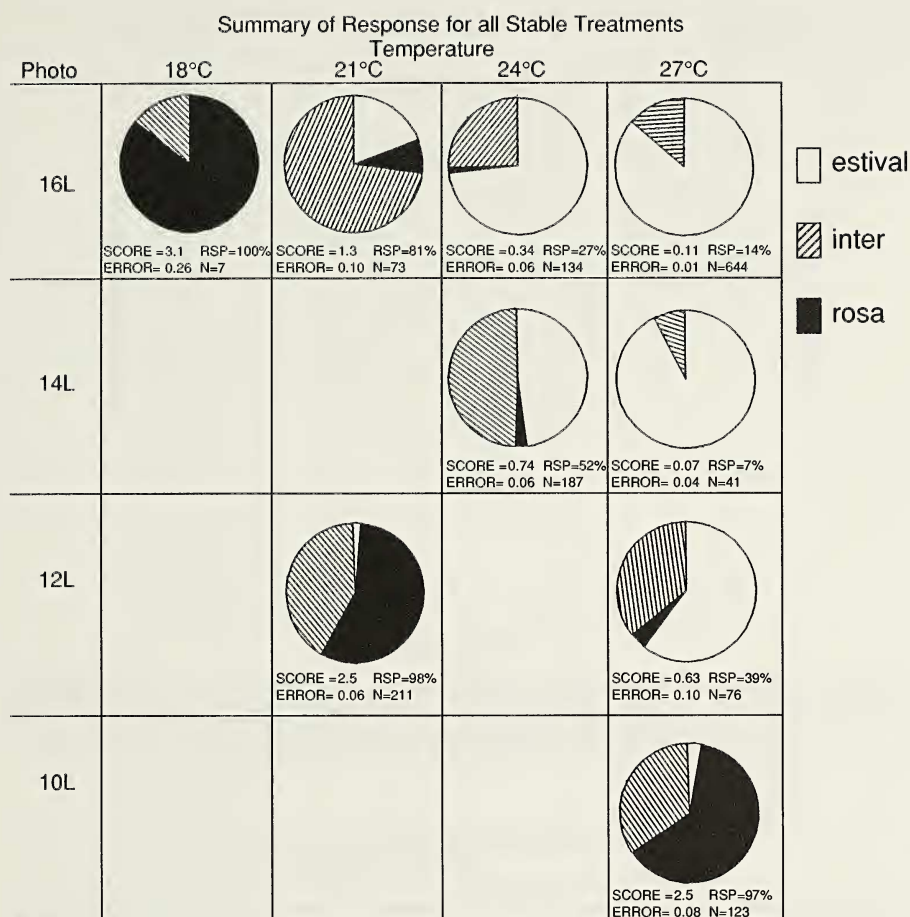
—— — Temperature / daylength

rosa morph (score > 2). Based on these findings, the response character-istics of the breeding colony (27°C and 16L:8D) were used as a baseline of comparison for other treatments.

SEASONAL CONDITIONS

Individuals reared outdoors under seasonal conditions showed a marked increase in both the incidence and degree of *rosa* correlated with short-ening daylengths and falling temperatures (Figure 2). The mean scores of animals raised under the conditions of the breeding colony (27°C, 16L:8D) and those raised outdoors under seasonal conditions remained quite similar from July through early August. However, by mid-August the responses began to diverge, with the population under seasonal conditions exhibiting a progressively higher incidence and degree of *rosa* coloration.

TEMPERATURE AND PHOTOPERIODIC REGIMES

While the seasonal exposures suggested that environmental cues might influence the onset and intensity of the *rosa* morph, the concurrent decreases in daylength and temperature made differentiation between

Summary of Response for all Stable Treatments
Temperature

SCORE = MEAN SCORE   RSP = % WITH INTERMEDIATE OR FULL RESPONSE
ERROR = STANDARD ERROR (SCORE)   N = SAMPLE SIZE

Figure 3. Summary of all temperature and daylength regimes: The extent and intensity of response for each of the nine controlled temperature and daylength regimes as shown by proportionately shaded pie charts. The percentage of *Linea* (score = 0) individuals is shown by the unshaded portions, intermediate individuals (score = 1 or 2) by the crosshatched portions and *Rosa* individuals (score = 3 or 4) by the completely filled portions. The top row shows response to different temperatures at the breeding colony daylength of 16L:8D while the rightmost column shows the response to different daylengths at the breeding colony temperature of 27°C. Below each piechart is additional information for that treatment: mean score and standard error, % response and sample size.

the effects of these two factors impossible. In order to study the effects of temperature and daylength separately, each was manipulated independently under controlled conditions in laboratory incubators. The results of all such manipulations are summarized in Figure 3.

The mean score and % response of animals subjected to progressively shortened daylengths rose sharply, even when temperature was held at
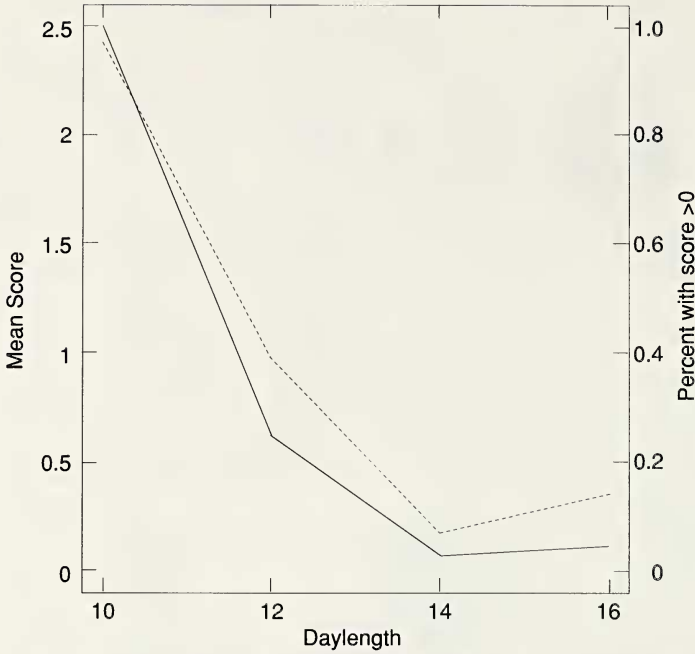
Figure 4. Response to daylength: The response characteristics of groups exposed
to different daylengths at the breeding colony temperature of 27°C. The
critical daylength (50 % response) lies between 10L:14D and 12L:12D at
27°C.

------ mean score
———— % response (% animals with score > 0)

the breeding colony value of 27°C (Figure 4). It was found that the critical
daylength (the daylength at which 50% of the population shows a
response) lies between 10L:14D and 12L:12D at 27°C. A comparable
increase in mean score and % response was noted in response to lowered
temperatures, even when daylengths were held at the breeding colony
value of 16L:8D (Figure 5). In this case, the critical temperature was
found to lie between 21°C and 24°C at 16L:8D.

Two balanced 2 X 2 matrices were constructed from the data in Figure
3: one including daylengths 14L:10D and 16L:8D with temperatures
24°C and 27°C, another including daylengths 12L:12D and 16L:8D with
temperatures 21°C and 27°C. Both matrices were subjected to 2 X 2
ANOVA analysis and each revealed highly significant temperature,
daylength and temperature-daylength interactive effects (P < 0.001).

RECIPROCAL TRANSFER STUDIES

Reciprocal transfers of larvae at various developmental stages were
carried out in order to investigate the critical period(s) for the induction
of the *rosa* morph. Larvae were staged into one of five developmental
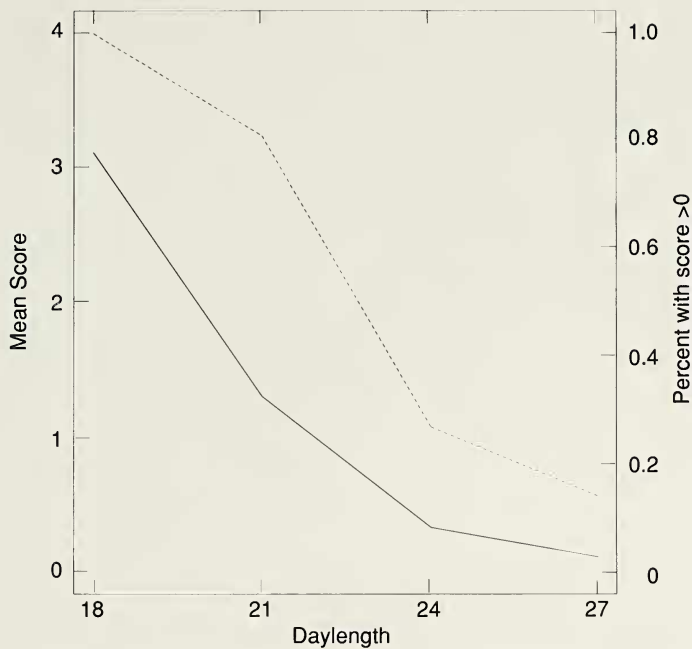classes: first instar, second instar, fifth (terminal) instar, pre-pupation

Figure 5. Response to temperature: The response characteristics of groups exposed to different temperatures at the breeding colony daylength of 16L:8D. The critical temperature (50 % response) lies between 18°C and 21°C at 16L:8D.

- - - - - -   mean score
————   % response (% animals with score >0)

and post-pupation. The pre-pupation class included those animals that were hanging by their anal prolegs in preparation for pupation (18-24 hours prior to onset of pupation). The post-pupation class included those animals in which pupation had occurred, but sclerotization of the pupal casing was not yet complete (approximately 1 hour after onset of pupation).

In one series of transfers, animals were removed from breeding colony conditions (27°C, 16L:8D) and subsequently reared under conditions known from previous experiments to produce a high incidence of the *rosa* morph (21°C, 12L:12D). The results of these transfers are summarized in Figure 6.

Animals transferred early in larval life (first instar) showed response characteristics very similar to those whose entire life was spent at 21°C, 12L:12D, indicating that the presence of temperature and daylength cues during egg and early larval stages of life is not crucial to the development of the *rosa* morph. Animals transferred in the interval near pupation - from 24 hours prior to pupation (fifth instar) to 1 hour post-pupation - showed a clear response to such conditions. However, the response was intermediate between the two untransferred control popu-
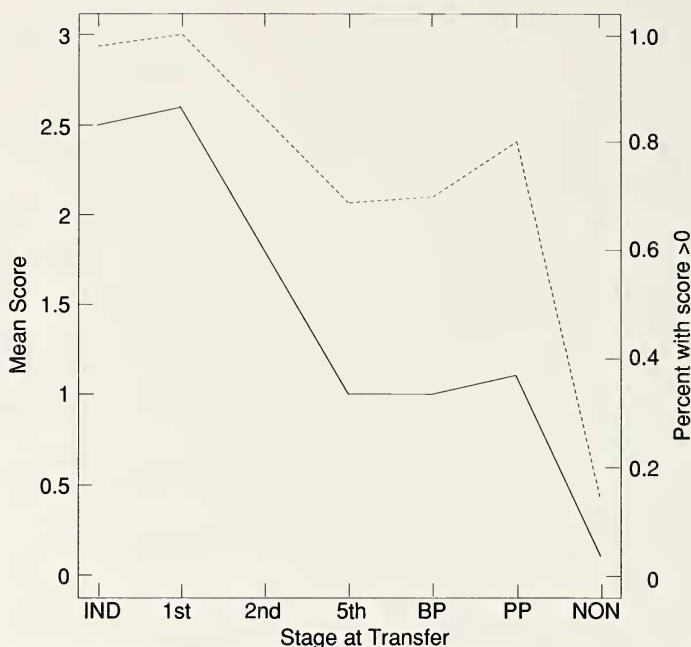
Figure 6. Response to transfer from conditions of the breeding colony: Response
  characteristics of animals transferred at various stages of development
  from the conditions of the breeding colony (27°C, 16L:8D) to conditions
  know to produce a high incidence and degree of *Rosa* coloration (21°C,
  12L:12D). Individuals transferred during the first instar (1ST) show a
  response comparable to the untransferred control population under con-
  stant conditions of 21°C, 12L:12D (IND). Individuals transferred during the
  fifth instar (5TH) show a response comparable to those transferred before
  pupation (BP) and post-pupation (PP). These are in turn intermediate
  between the untransferred breeding colony (NON) and 21°C, 12L:12D
  (IND) control groups, indicating both a cumulative process of *Rosa* induc-
  tion and a heightened sensitivity to conditions near pupation.
- - - - - - mean score
───────── % response (% animals with score >0)

lations. The fact that their response characteristics are significantly
lower than that of animals reared at 21°C, 12L:12D for most or all of
larval life suggests that larval conditions exert a cumulative effect. On
the other hand, the fact that their response characteristics are signifi-
cantly higher than that of animals raised exclusively under the condi-
tions of the breeding colony suggests a relatively high sensitivity to
conditions at or near pupation.

A series of reciprocal transfers was also conducted in which individuals
reared at 21°C and 12L:12D were transferred to the conditions of the
breeding colony (27°C, 16L:8D) at various stages of development. The
results of these transfers are summarized in Figure 7.

Again, animals transferred to the conditions of the breeding colony
very early in larval life (first instar) exhibited response characteristics
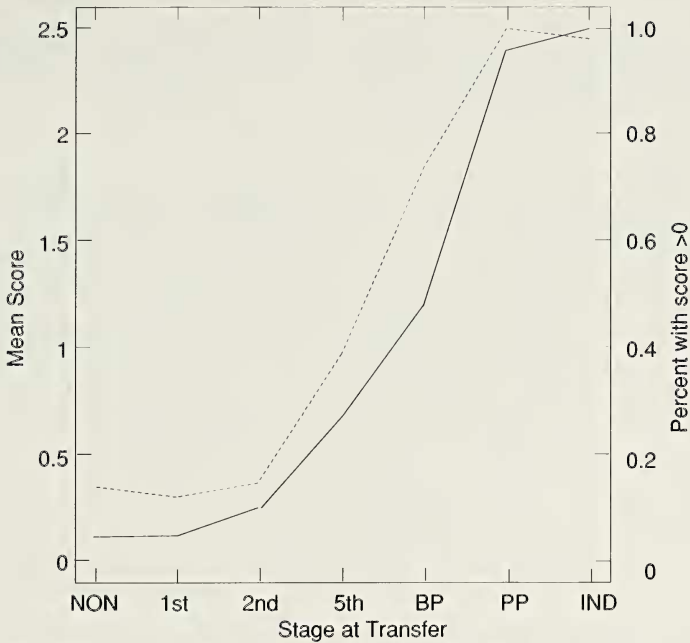
Figure 7. Response to transfer to conditions of the breeding colony: Response characteristics of animals transferred at various stages of development from conditions know to produce a high incidence and degree of *Rosa* coloration (21°C, 12L:12D) to the conditions of the breeding colony (27°C, 16L:8D). Individuals transferred during the first instar (1ST) show a response comparable to the untransferred breeding colony control at 27°C, 16L:8D (NON). Individuals transferred during the second instar (2ND) retain some residual response as compared to the breeding colony control (NON) despite being exposed to conditions of 21°C, 12L:12D for only a few days longer than first instar transfers (1ST). Individuals transferred during the fifth instar (5TH), before pupation (BP) and post-pupation (PP) show a progressively stronger retention of the *Rosa* response. Note the divergence in response between individuals transferred before pupation (BP) and those transferred post-pupation (PP), despite a difference in exposure to the 21°C, 12L:12D conditions of only 19-25 hours. This indicates a rapid "fixation" of the *Rosa* response near pupation. Moreover, the retention of response by post-pupation transfers (PP) at a level comparable to the untransferred 21°C, 12L:12D control population (IND) indicates a complete fixation of the response within an hour of pupation.

- - - - - - mean score
———— % response (% animals with score >0)

indistinguishable from the breeding colony control population. However, those transferred only a few days later (second instar) seem to retain a slightly heightened response. Animals transferred later show progressively stronger retention of response characteristics, with those transferred in the post-pupation stage retaining response characteristics indistinguishable from the untransferred control population at 21°C, 12L:12D. Of special interest is the fact that the post-pupation transfers

show markedly stronger response characteristics than animals transferred immediately before pupation, even though an interval of only 19-25 hours separates the two stages.

Discussion

Short daylengths have been shown to trigger the appearance of the *rosa* morph in *Precis coenia*. This is not surprising since many, if not most, insects respond in some fashion to photoperiodic cues (Beck, 1980; Tauber et. al, 1986). In particular, photoperiodic cues are thought to predominate in the polyphenisms of four families of Lepidoptera, including the Nymphalidae (Shapiro, 1976; Brakefield & Larsen, 1984).

Low rearing temperatures alone seem to be as effective as short daylengths in producing the *rosa* morph. While temperature is a common modifier of photoperiodic induction, examples of systems with true multiple induction are relatively rare. In particular, although multiple induction systems have been shown to exist in other insects (e.g., aphids), the only other clearly documented example among the Lepidoptera is by Shapiro (1982) - although there is also some evidence that the related species *Precis octavia* and *Junonia villida* may have similar induction patterns (McLeod, 1968; James, 1987).

The reason for multiple induction in the *rosa* system is unclear. In general, it is thought that photoperiodic cues are more reliable indicators of seasonal change than highly variable temperatures. Thus, it is often argued that only in areas where reliable photoperiodic cues are unavailable (e.g., equatorial regions and foggy montane habitats) will reliance on temperature cues evolve (Beck, 1980; Shapiro, 1984; Tauber, et. al., 1986). For example, as an equatorial species *P. octavia* is exposed to a daylength with little seasonal variation, so the appearance of a temperature-regulated mechanism for the polyphenism is easily explained. But *P. coenia* is a temperate species and is subjected to seasonal daylength fluctuations of more than 3 hours in North Carolina - ample range for the development of a predominantly photoperiodic induction if such a cue were inherently superior to temperature.

Shapiro (1978) has suggested that seasonally polyphenic systems in general should tend to have redundant induction mechanisms. If so, the dearth of examples in the literature may be due to an overzealous focus on the importance of photoperiodic cues. On the other hand, Shapiro has also argued (1976) that seasonal polyphenisms are character traits which represent a high degree of phylogenetic information. If it turns out that multiple induction of seasonal polyphenisms is relatively rare, then perhaps the *rosa* system represents an early stage in the evolutionary transition from temperature to photoperiodic control in a species with a tropical ancestry.

The reciprocal transfer studies seem to indicate that the induction of the *rosa* morph is a cumulative process beginning early in larval life, accelerating during the terminal larval instar and culminating within 1

hour of the onset of pupation. The protracted nature of *rosa* induction is unusual since the majority of insects respond to seasonal cues only during a tightly defined critical period, although there other are examples of cumulative induction patterns - as in *Pectinophora gossypiella* and *Ostrinia nubilalis*, as well as in the pierid butterflies *Pieris rapae* and *Pieris brassicae* (Beck, 1962; Barker, et. al., 1963; Shapiro, 1968).

The complexity of the *rosa* polyphenism may help explain why elucidation of its seasonal cues has so long eluded researchers. Although the present study solves this particular puzzle, it raises many new ones to take its place. Obvious questions remain concerning the exact physiology of the system and these are the focus of ongoing research. Perhaps most intriguing however, are questions concerning the evolutionary ecology of *rosa*. There is interesting evidence that the *rosa* system has a strong genetic basis and exhibits clinal variation in the wild (Smith, 1991) but the adaptive significance (or insignificance) of the *rosa* morph is unknown, as are many other parameters of evolutionary importance such as migration and gene flow patterns. Klots' (1951) observation concerning the Buckeye is just as true today as it was 40 years ago: "A great deal of careful work and thorough analytical study is needed."

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